

## ORGANIC PRODUCTIVITY IN THE REPRODUCTIVE CYCLE OF THE PURPLE SEA URCHIN <sup>1</sup>

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The gonads of a gravid purple sea urchin (*Strongylocentrotus purpuratus*) may contribute as much as one-fifth to the total wet weight of the animal. On the other hand, the shrunken gonad of an immature animal or one which has recently spawned may be only one-eighteenth as large. The development of the gonad represents a remarkable synthesis of organic material, since the larger part of the protoplasm of a sea urchin is gonadal during the breeding season, the only other organ of any bulk being the intestine. The intestine in turn owes part of its bulk to its food contents, the epithelium itself being quite delicate. The volume of perivisceral fluid bears an inverse relation to the gonads, being present in larger amounts when the gonad is less well developed. The perivisceral fluid, however, contains little organic material (Lasker and Giese, 1954). Furthermore, its organic constituents do not vary in any striking or systematic way during the year (Bennett and Giese, 1955). A fairly good measure of organic productivity in the sea urchin might therefore be gained by a study of the increase in organic constituents in the gonads during their growth from immature (or spent) to gravid condition. The results of such study are reported in this paper.

### METHODS

For most of the experiments reported here, sea urchins were collected at the monthly low tide at Yankee Point, near Carmel, California. In a few instances specimens were obtained near Moss Beach, California. The gonad index was determined for each of ten specimens, the index being the ratio of the volume of gonad to wet weight of animal, times 100. The total nitrogen (TN), non-protein nitrogen (NPN), lipid, and glycogen contents of samples of gonad were determined. For one male and one female, water and ash content of the sample were also determined monthly. From samples at the height of the season, and also after the spawn-out, determinations were made of the desoxyribonucleic acid (DNA) and ribonucleic acid (RNA), as well as lipid, total nitrogen and non-protein nitrogen at the same time, and in a few samples reducing sugar (RS) content was determined. These data give a biochemical picture of the constituents of the gonads correlated with the gonadal cycle over an entire year.

For the biochemical determinations on the gonads of each animal, several samples

<sup>1</sup> Supported by funds made available by National Science Foundation Grant GS 482, U. S. Public Health Grant 4578, and the Rockefeller Foundation. We are indebted to Mr. F. Falconer, head librarian of the Biological Libraries, for verification of the literature cited.

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of various wet weights (several grams) were placed in a vacuum desiccator over concentrated sulfuric acid and dried for about 12 hours. When tissues were to be used for glycogen analysis, a few drops of 10 per cent trichloroacetic acid were injected before drying to prevent glycolysis by enzymes during the drying process. All analyses were done in duplicate; the duplicates varied by only a few per cent.

For determination of total nitrogen, a given sample was digested in sulfuric acid with selenium catalyst over electric heat or gas flame, and from an aliquot of the digest the ammonia was distilled with a Markham still (Markham, 1942), or in a Conway diffusion cell (Conway, 1947), into borate buffer containing brom-cresol green and methyl red as indicators. The borate was then titrated to the original color with 0.01 N sulfuric acid. Usually several weights of samples were tested and to one of them a known weight of a nitrogen-containing compound (glycine) was added to serve as a check on the accuracy of the method.

For determining non-protein nitrogen 1 ml. of 10 per cent trichloroacetic acid (TCA) was added to a 10–30 mg. sample of gonad and the tissue was macerated with a glass rod. It was heated to 80–100° C. in an oven for 15 minutes, allowed to cool, centrifuged, and the supernatant plus two washings of the precipitate were added to the flask which was then placed on the digestion rack and the nitrogen content determined as described above. The non-protein nitrogen subtracted from the total nitrogen is taken to give the protein nitrogen (PN)<sup>3</sup>. This is multiplied by the factor 6.25 to convert to protein.

Total lipids in gonadal tissue were determined by extracting 100-mg. samples with 10 ml. ethyl ether in a micro-Soxhlet apparatus, refluxing being continued for two hours. Many samples were extracted at the same time on a sand bath.

Glycogen was determined in the following way (Good *et al.*, 1933; Meyer, 1943). The ground dry sample was treated with an equal volume of 10 per cent TCA, cooled, and the supernatant was transferred to a lusteroid tube to which was added 1 ml. of distilled water wash of the precipitate. After addition of 2.5 ml. of 95 per cent ethanol and mixing, the sample was centrifuged and the supernatant fluid was discarded and the tube allowed to drain for several minutes. To it was added enough warm water to give about 70  $\mu$ gm glycogen per ml. and the content of glycogen was determined by the anthrone method (Seifter *et al.*, 1950). Reducing sugar was determined in the supernatant fluid of a homogenized gonad by the Somogyi method (1945; 1952) which involves first the precipitation of the protein by TCA, centrifuging the sample, and testing of the supernatant solution.

Water content was determined by weighing minced tissue before and after drying in the desiccator over sulfuric acid. Ash content was determined on a known dry weight of gonad (about 100 mg.) heated to 450–500° C. in a porcelain crucible for three to eight hours.

Nucleic acids were extracted using the Hershey, Dixon and Chase (1953) adaptation of the Schmidt-Thannhauser (1945) and Schneider (1945) procedures, acid-soluble phosphorus being removed by cold 10 per cent TCA, phospholipid being re-

<sup>3</sup> Although it is classical procedure, some question exists whether this is entirely justified here, because when a direct test for protein nitrogen is made on the residue remaining after extracting acid-soluble phosphates, phospholipids and nucleic acids from the tissue mash, only about a half to a third as much is obtained as by the difference between total nitrogen and non-protein nitrogen. It is possible that some of the proteins are dissolved by the extraction procedures, but additional studies are desirable.

moved with ethanol and a mixture of ethyl ether and ethanol (60° C.). RNA was removed with KOH, DNA being precipitated with 5 per cent TCA (Leslie, 1955). The indole reaction of Ceriotti (1952) was used for DNA and the orcinol reaction of Ogur and Rosen (1950) was used for RNA; the details of the method as used here have been described elsewhere (Iverson and Giese, 1957). Some studies were made determining the nucleic acids by the phosphorus method (Fiske and Subbarow, 1925) but they were considered less reliable and are not reported here.

## RESULTS

The average values for some chemical constituents of gonads of male and female sea urchins taken each month of the year 1956 are given in Table I. Certain trends

TABLE I

*Chemical constituents of gonads of the purple sea urchin (Jan. to Dec. 1956)*  
(Water in % wet weight, all others in % dry weight)

Date	Av. GI*		Lipid		NPN		Protein		Glycogen		Water		Ash	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
1/25	9.2	7.5	10.0	13.6	1.4	1.2	44.9	31.5	5.9	10.8	65.1	67.0	—	—
2/21	7.6	7.2	20.5	22.0	3.8	3.0	34.2	30.2	5.7	6.6	69.7	71.3	7.1	7.0
3/31	4.4	3.0	12.9	19.4	3.2	2.1	30.7	27.7	3.0	10.2	74.7	77.8	9.5	8.1
4/20	3.5	1.8	19.5	16.1	3.1	3.0	31.1	24.1	14.0	4.1	78.1	76.2	7.4	4.8
5/30	5.9	5.7	14.5	15.4	3.0	2.3	27.0	27.9	10.6	10.3	71.1	58.5	—	2.6
6/17	3.8	4.6	19.8	19.8	2.4	2.0	22.7	23.2	5.2	7.1	70.1	61.3	4.7	2.4
7/31	5.6	10.0	18.5	19.0	2.3	1.8	26.3	26.4	5.7	5.2	68.2	55.0	1.9	2.2
8/30	6.7	6.7	16.3	13.2	2.4	2.6	21.5	18.4	1.0	1.0	63.2	74.3	3.7	2.7
9/27	12.4	15.5	15.5	18.7	2.4	1.8	34.0	26.3	1.6	1.9	68.7	64.9	5.5	3.5
10/31	11.9	12.8	22.4	21.2	2.8	1.5	35.2	35.4	3.4	3.9	70.0	73.5	3.2	2.7
11/28	14.0	14.4	10.5	15.9	2.0	2.0	33.4	35.1	7.8	6.9	69.0	67.0	5.3	4.4
12/18	17.5	16.6	15.9	20.1	3.7	2.3	36.2	39.8	3.2	3.6	66.1	66.3	7.1	5.8
Av.			16.4	24.5	2.7	2.1	31.5	29.0	4.8	6.0	69.5	67.7	4.6	3.8

\* GI refers to gonad index obtained as defined in the text. NPN refers to non-protein nitrogen.

appear in the data of this table. At times of the highest gonad index, the gonads per unit weight tend to contain more lipid, protein, glycogen and ash and less water (especially in the female) than at the time of low gonad index. A more significant rendition of the data of Table I is given in Figure 1, because it shows the distribution of each chemical in gonads of members of a population sample taken each month. It will be observed that at all times of the year gonads of some individuals of a population sample may have relatively large amounts of certain constituents, while gonads of other individuals of the same population sample may have a relatively small amount. Certain trends do appear but an average value which emphasizes these trends gives a less true picture of the actual facts than the distribution plot. Statistics calculated from the data are not a truthful representation of the data, because standard deviations and confidence limits are meant to apply to a population

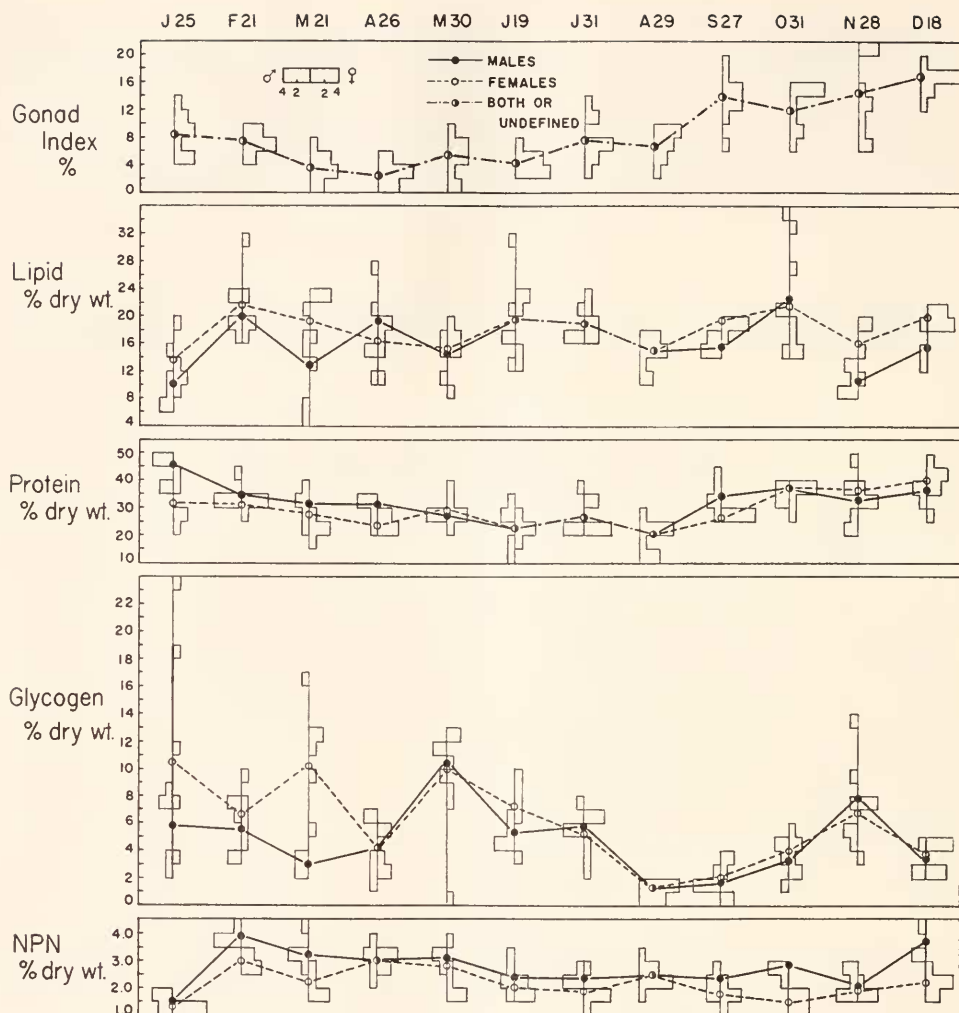


FIGURE 1. The distribution graphs illustrate the inhomogeneity of the population of sea urchins throughout an annual cycle, not only in gonad index but in content of various organic constituents in the gonads (last four graphs). Glycogen content of gonads shows greatest variability, non-protein nitrogen (NPN), least. For explanation see text.

with a normal distribution, not to a skewed one such as is the population dealt with here.

The over-all averages for the entire year disclose some interesting information about the gonads (last line, Table I). The ovary is distinctly richer than the testis in lipid and glycogen but the testis appears to be richer than the ovary in non-protein nitrogen, protein nitrogen and possibly in salts (ash) and water, although the few samples taken and their variability from month to month make any deductions on the latter two substances questionable.

When a sample of animals is selected and the ones with a low gonad index are compared with those with a high index, the contrasts in chemical constitution of gonads during the course of the reproductive cycle are most clearly brought out as seen in Table II. In addition to the chemicals discussed above, it is seen that the RNA per unit weight of the ovary increases with its enlargement while the DNA decreases; in the testis the reverse is true, the RNA per unit weight decreases while the DNA more than doubles.

These differences between ovary and testis are understandable in view of the

TABLE II

*Chemical constituents in spent and gravid gonads of the purple sea urchin (in % dry wt.)*

Sex and condition	Gonad index	NPN	Protein	RNA	DNA	TN	Reducing sugar
♂ spent	2.3	2.0	23.1	3.25	0.0014	5.7	0.007
	—	2.0	27.4	3.46	0.0010	6.4	
	3.5	2.2	23.7	2.59	0.0018	6.0	
Av.	2.9	2.1	24.7	3.10	0.0014	6.0	
Gravid	17.8	1.3	35.0	5.0	0.0007	6.9	0.037
	18.2	2.1	34.4	4.8	0.00065	7.6	
	18.9	1.0	39.4	3.8	0.00059	7.3	
Av.	18.3	1.5	36.3	4.5	0.00065	7.3	
♂ spent	1.52	4.1	25.9	2.3	4.7	8.3	0.0036
	2.84	2.1	36.0	2.4	4.1	7.9	
	2.43	2.2	36.0	2.1	4.4	8.0	
Av.	2.26	2.8	32.6	2.3	4.4	8.1	
Gravid	21.5	0.66	43.0	1.3	9.8	7.6	0.034
	19.2	1.20	39.8	0.9	8.0	7.6	
	1.77	0.59	42.5	0.9	9.3	7.4	
Av.	19.5	0.82	41.8	1.0	9.0	7.5	
		NPN	Protein	Lipid	Glycogen	TN	Water
♀ spent	1.18	3.75	23.2	9.5	0.41	7.5	63.2
	1.28	3.07	20.1	18.7	3.35	6.3	78.8
	1.49	2.84	28.2	15.5	2.77	7.4	—
Av.	1.32	3.22	23.8	14.6	2.18	7.1	71.0
Gravid	21.8	1.83	33.5	18.1	7.98	7.2	—
	17.0	1.92	26.8	19.9	2.35	6.2	64.9
	17.7	2.41	42.1	21.3	4.54	9.1	70.0
Av.	18.8	2.05	34.1	19.8	4.96	7.5	67.4
♂ spent	1.43	3.03	38.2	17.4	2.63	9.2	—
	3.0	3.14	33.4	14.2	6.41	8.5	76.2
	1.42	3.34	32.4	20.5	1.48	8.5	74.9
Av.	1.95	3.17	34.7	17.4	3.51	8.7	75.5
Gravid	21.3	1.63	37.5	13.0	4.24	7.9	—
	21.0	1.91	24.4	11.0	9.86	5.8	65.1
	21.6	4.47	35.2	12.6	4.43	10.1	66.3
Av.	21.3	2.67	32.4	12.2	6.51	7.9	65.7

gametes produced and their prominence in the gravid gonads. It will be remembered that female sea urchins can usually be distinguished from male sea urchins during all months of the year by the presence of eggs in the ovary, even though the eggs may be small and immature. Only occasional specimens are indeterminate as to sex, either just after spawn-out or because they have not yet matured (very small ones, that is, less than 17 mm. in test diameter are always indeterminate for the latter reason). Conversely, males can usually be detected by the presence of sperm in the testis. Eggs contain considerable stores of food for the development of the embryo while sperm contain only stores for the brief period of locomotion of the sperm preceding fertilization. *A priori*, one expects eggs to be rich in lipids and glycogen, whereas sperm are expected to contain some glycogen as food reserve for movement. One also expects the eggs to contain more RNA than sperm but less DNA. As can be seen from the data, these expectations are indeed realized. More suprising is the fact that the spent or immature gonads also show contrasts in chemical con-

TABLE III

*Increase in organic constituents of gonads of the purple sea urchin during growth from shrunken to maximal size (in mg.; total wt. in grams)*

	♂		Relative increase	♀		Relative increase
	Spent	Gravid		Spent	Gravid	
Gonad index	1.42	21.6	15.2 ×	1.18	21.8	18.5 ×
Total wt. (arbitrary) in grams	1.0	15.2	15.2 ×	1.0	18.5	18.5 ×
Total nitrogen, mg.	84	1170	14.0 ×	65.5	1369	21.0 ×
Non-protein nitrogen, mg.	29.9	266	8.9 ×	26.6	328	12.3 ×
Protein, mg.	336.5	5639	16.7 ×	243	6512	26.7 ×
Lipid, mg.	174	1854	10.4 ×	146	3663	25.1 ×
Glycogen, mg.	35	989	28.2 ×	21.8	917.6	42.1 ×
Reducing sugar, mg.	0.036	5.16	143.0 ×	0.07	6.84	98.0 ×
RNA, mg.	23	152	6.6 ×	31.0	832.5	26.8 ×
DNA, mg.	44	1368	31.1 ×	0.014	0.120	8.6 ×

stitution, especially the large lipid content of immature or shrunken ovaries as compared to immature or shrunken testes. Presumably the lipids are present in the ovarian epithelium which gives rise to the eggs. Histochemical studies would be interesting on ovarian and testicular materials at different times in the gonadal cycle.

It is not possible to ascertain productivity of organic materials in the gonads of the sea urchin on a per unit weight basis, because all that is then observed is a shift in emphasis on certain materials, which accompanies the onset of maturity, *i.e.*, a synthesis of some materials at a greater rate than that of others. Furthermore, the relative content of water in the ovary declines to some extent concomitantly with a general increase in the total mass of other substances in the ovary. Therefore, to ascertain organic productivity of the gonads it is necessary to take into consideration the increase in mass of the gonads, as well as their change in chemical constitution (per unit weight) during the growth from a spent to a fully gravid con-

dition. Gonads increase in mass by a ratio which equals the gonad index of a gravid animal divided by the gonad index of a spent animal. For a female this is 18.5-fold, for a male it is 15.2-fold (using the data for maximal and minimal sizes of gonads given in Table II). If the gonads of a spent animal weigh 1 gram, as they would in fact for an average-sized animal of 90 grams total wet weight, then the ovaries of a gravid female of this size would weigh 18.5 grams and the testes of a gravid male of this size would weigh 15.2 grams. The content of each chemical constituent in the spent and gravid gonads of animals of this size could then be calculated by multiplying the weight of the gonad in grams by its per cent content of each of the constituents given in Table II. Data so calculated are given in Table III. By dividing the content of each constituent in the gonad of a gravid individual by the content of that constituent in the gonad of a spent animal, the relative increase in mass of the chemical constituent in question during the growth of the gonads from the spent to the gravid state was calculated and the data are given in Table III. For example, to obtain the content in NPN in a spent ovary its weight, 1000 mg., is multiplied by the average fractional content<sup>4</sup> of NPN in spent ovaries, 2.66 per cent or 0.0266, giving 26.6 mg. To calculate the NPN in a gravid ovary its weight, 18,500 mg., is multiplied by the average fractional content of NPN in gravid ovaries—1.775 per cent or 0.01775. This gives a value of 328 mg. The increase in mass of NPN from spent to gravid condition is then 328 divided by 26.6, which is 12.3 times.

The chemical constituents showing the most striking total increases during the growth of the gonad observed in Table III are of course the ones which have also increased on a per unit weight basis. It will be seen that the total amount of DNA in the testis increases by about 31 ×, the RNA in the ovary 27 ×, the glycogen in the testis 28 ×, the glycogen in the ovary 42 ×, the lipid in the ovary 25 ×, the lipid in the testis 10 ×, the protein in the testis 17 ×, the reducing sugar in the testis 143 × and in the ovary 98 ×.

## DISCUSSION

It is interesting at this time to inquire about several matters concerning the gonadal biochemical cycle in the purple sea urchin. To what extent is it possible to explain the chemical diversity in gonads in a population of sea urchins selected at random at any time during the year? How does the build-up of the nutrients in the gonads occur? What is the over-all productivity of the purple sea urchin?

The variability of chemical constitution of the gonads of the sea urchin during the year may be just another index of the failure to get synchronized spawning in this species. At almost all times the population is rather inhomogeneous with respect to the gonad cycle, some animals having fairly well-developed gonads while others are poorly developed or spent. Only in March and April is the gonad index rather low for most specimens and only in December is it consistently high. Biochemical inhomogeneity of different individuals may therefore reflect population inhomogeneity in gonadal development. Even when animals of like gonad index are compared, however, one finds biochemical differences. Perhaps an individual just spending or one just building up to the same intermediate gonad index, may be

<sup>4</sup> The average of the values for the two groups of spent animals in Table II, namely, 2.1 and 3.2 per cent, giving 2.65 per cent or 0.0265.

quite different histologically and histochemically. Information on this as a possible explanation of chemical inhomogeneity is lacking at the present time.<sup>5</sup>

Another factor which may play a role in the variability in chemical constitution of the sea urchin gonad is availability of nutrients at different times during the year, or at any one time, a difference in availability of nutrients to each individual in the population. The relative immobility of the urchins which have bored their way into the soft rocks makes them dependent upon what grows in their immediate vicinity or what the waves may bring to them by chance. The gonad is the main storage organ of the sea urchin, a little organic material also being stored in the gut (Hilts and Giese, 1949). When an urchin is starved the gonad shrinks and its gonad size may decline even without spawning. However, the intestines of almost all urchins from the field are filled with algae; therefore food seems to be generally available. The purple sea urchin's willingness to eat almost any food, animal or plant, when starved, makes it seem unlikely that it lacks in quantity of food in nature. However, the food may have unequal nutritive quality at different times. No evidence was collected upon this point, but young growing algae are known to contain much protein while old ones are made up, to a considerable extent, of polysaccharides which are probably a much less available source of food (Wort, 1955). The availability of nutrients may therefore vary even though the bulk of food taken in may be the same.

The build-up of nutrients in the gonads must be a relatively slow process, yet the increase in organic matter during a gonadal cycle is rather striking, indicating effective digestion, mobilization, and conversion of food. Digestion appears to be a rather slow process in the sea urchin, since algae may be defecated for several weeks from a single gutfull in an animal deprived of further sources of food. While the enzymes of the sea urchin readily handle proteins and starch, they attack few of the polysaccharides of the algae (Lasker and Giese, 1954; Huang and Giese, 1958). However, bacteria may play a role in digestion since they readily hydrolyze the algal polysaccharides in the gut of the urchin. Where the nutrients go when they leave the intestine is not clear. The perivisceral fluid contains some protein, reducing sugar, lipid and very little non-protein nitrogen. Most of the protein forms striking fibrous clots. When these are filtered out the remaining fluid appears to be protein-free (TCA negative).<sup>6</sup> It is possible that the continual dribble of sugar, amino acids, and possibly lipids, from the intestine into the body fluid, is adequate for the build-up of the reserves in the gonads. However, it is desirable that someone explore other pathways of nutrient transport, particularly by wandering amebocytes and by the haemal system which extensively vascularizes both the gut and the gonads (Hyman, 1955).

To assess the over-all productivity of the sea urchin it is necessary to consider not only the gonad cycle and the increase in organic material which occurs there, but also other possible constituents which accumulate organic materials. The only

<sup>5</sup> That the small size of the sample of the population is not the cause of the variability of the gonads is shown by a study with larger sample sizes by Josef Miller of Monterey Peninsula College. He compared the gonad index of samples of 10, 20, 40 and 80 sea urchins. The gonad index for a given population of sea urchins at a given season was almost the same, within a few per cent, regardless of the sample size.

<sup>6</sup> However, two protein peaks are disclosed in paper electrophoresis studies of fluid filtered after clotting (Favour and Giese, unpublished).

organ of considerable size in the sea urchin other than the gonad is the intestine, but some tissue is also present in the water vascular system, the muscles of the spines and pedicellariae, the dermal branchiae, the epidermis, the mesenteries, and the coelomic lining. In an urchin of about 90 grams, all of these structures are estimated to weigh about 7 grams.

If, for purposes of argument this figure is tentatively accepted, then the total increase in organic material with one gonadal cycle is approximately three-fold. Unfortunately we do not know how many gonadal cycles a single sea urchin can undergo in one season. The fact that a population of sea urchins collected at almost any time of the year, with the exception of the time of the highest gonad index and the period just after the maximal spawn, shows individuals with widely different indices (see Figure 1 and the figures in Bennett and Giese, 1955), suggests that a single individual may spawn several times during the year. If this is true, several times the above figure may be a more nearly correct estimate of production of organic material. Since the sea urchin also grows in diameter and bulk, the true figure must be larger on this account as well. We do not at present have sufficient data to make a determination of the growth rate and the rate of incorporation of nutrients into body material.

#### SUMMARY

1. Monthly determinations were made of the amount of lipid, glycogen, non-protein nitrogen, protein, water, and ash present per unit weight in gonads of the purple sea urchin, *Strongylocentrotus purpuratus*. Tests for reducing sugar, DNA and RNA were made for gonads at the height of the reproductive season and after spawning-out.

2. A change in relative proportions of the chemical constituents was observed with maturation of the gonads. In the ovary protein, lipid, glycogen, reducing sugar and RNA increase proportionally more than the over-all increase in bulk of the gonad, while DNA and possibly water, increase proportionally less. In the testis, glycogen, reducing sugar, DNA and possibly protein, increase proportionally more than the over-all increase in bulk, while RNA, lipid, and possibly water, increase less than the increase in total bulk.

3. A considerable increase in the total amount of all the organic constituents tested here occurs during the growth of gonads. Thus, a gravid ovary is about 18.5 times the bulk of a spent one and a gravid testis is about 15.2 times the bulk of a spent one.

4. The sources of nutrients and the possible transport are discussed with reference to the literature.

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